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Short communication

Mutagenic evaluation of 10 long-term stored medicinal plants commonly used in South Africa

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ABSTRACT

The use of medicinal plants is an increasing phenomenon among the majority of people in many developing countries. Some of the harvested medicinal plants are often stored for shorter or longer periods prior to usage. Evidence from recent studies has demonstrated the pharmacological efficacy of short and long-term stored plant materials when compared to freshly-harvested ones. In an attempt to evaluate the effect of long-term storage on the safety of some commonly used medicinal plants, the Ames test which involved the use of three *Salmonella typhimurium* tester strains (TA98, TA100 and TA1535) were conducted. Current findings indicate the absence of any mutagenic effects resulting from the storage of medicinal plant materials for as long as 16 years. Although freshly collected *Acokanthera oppositifolia* extract demonstrated a mutagenic effect against TA1535 strain at the highest concentration tested, no such effect was observed in the stored material. Further studies involving metabolic activation systems and *in vivo* conditions may further elucidate the effect of long-term storage on the safety of medicinal plants.

1. Introduction

Globally, the increasing importance and potential of medicinal plants remain evident (Newman et al., 2003; Gurib-Fakim, 2006). In view of the numerous benefits associated with the use of medicinal plants, it has become widely accepted and now forms an integral part of the culture in many developing countries including South Africa (Mander et al., 2007). Much literature highlighting the rich biodiversity and the popularity of medicinal plants in South Africa is available (Hutchings et al., 1996; Mander et al., 2007; Van Wyk, 2011). Over the past two decades, certain critical aspects of medicinal plant research including their efficacy, conservation and safety have received attention (Fennell et al., 2004a,b; Verschaeve and Van Staden, 2008; Rybicki et al., 2012). Presumably, the availability of such vital information will be valuable in the formulation of effective policies geared towards ensuring the successful integration of traditional medicine into a public health framework (Alves and Rosa, 2007; Street et al., 2008).

In an attempt to alleviate the problem of overexploitation and rapid depletion of plant biodiversity due to the heavy reliance on plants collected from the wild by a burgeon of informal city dwellers for therapeutic remedies, different approaches such as the cultivation and proper storage of medicinal plants have been recommended (Fennell et al., 2004a; Wiersum et al., 2006). In addition, there is the need for more research geared towards the better understanding of the post-harvest physiology of medicinal plants (Fennell et al., 2004a). This is due to concerns regarding the pharmacological efficacy of medicinal plants after an indefinite period of storage. There is a possibility that post-harvest processes may cause chemical changes in the plants due to the effects of oxidising enzymes, environmental and storage conditions (Fennell et al., 2004a; Stafford et al., 2005). Therefore, it becomes pertinent to investigate the effect of storage on the pharmacology and toxicology of medicinal plants. A number of researchers have critically evaluated the effects of storage on the dynamics of plant secondary metabolites and the pharmacological efficacies of medicinal plants (Griggs et al., 2001; Stafford et al., 2005; Amoo et al., 2012a,b). In most cases, the authors disclosed that medicinal plants could retain their pharmacological potency after different periods of time. Nevertheless, there is a dearth of information, if any, on the safety of medicinal plants after prolonged storage. Consequently, the present study evaluated the potential mutagenic effects of 10 commonly used South African medicinal plants which were stored for 12 or 16 years in comparison to the freshly collected plant materials.

2. Materials and methods

2.1. Plant material collection and extract preparation

The list of plant species, plant parts, places of collection and voucher specimen numbers of freshly harvested plants evaluated in this study is shown in Table 1. The traditional medicinal usages of the selected plant species, voucher specimen numbers of the stored material and their places of collection have been documented in previous studies (Jäger et al., 1996; McGaw et al., 2000). In order to eliminate environmental

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Table 1
Mutagenic activity of 10 long-term stored South African medicinal plants in terms of number of histidine (his^+) independent revertants per plate using three *Salmonella typhimurium* tester strains.

Family	Plant species	Plant part(s)	Place of collection	Voucher number	Conc (μg/ml)	TA98		TA100		TA1535	
						Stored	Fresh	Stored	Fresh	Stored	Fresh
Anacardiaceae	*Protorhus longifolia* (Bernh.) Engl.	Leaves	SNR	S. Amoo 19 NU[a]	50	15 ± 2.4	17 ± 1.2	191 ± 9.5	173 ± 16.7	16 ± 2.0	16 ± 3.2
					500	16 ± 1.5	17 ± 2.8	197 ± 22.2	161 ± 4.6	14 ± 3.2	12 ± 0.3
					5000	20 ± 3.5	18 ± 2.0	185 ± 14.3	169 ± 12.3	12 ± 1.7	16 ± 2.1
Apocynaceae	*Acokanthera oppositifolia* (Lam.) Codd	Roots	SNR	A. Aremu 1 NU[a]	50	19 ± 2.7	20 ± 3.0	153 ± 12.7	150 ± 5.5	14 ± 2.0	16 ± 2.9
					500	21 ± 1.0	20 ± 3.5	163 ± 12.9	129 ± 3.2	14 ± 2.1	27 ± 16.7
					5000	20 ± 2.0	36 ± 6.7	187 ± 6.2	120 ± 3.5	12 ± 1.0	184 ± 71.1
Asteraceae	*Artemisia afra* Jacq. ex Willd	Aerial parts	UKZN	S. Amoo 15 NU[a]	50	18 ± 3.2	18 ± 1.7	154 ± 12.3	155 ± 12.9	17 ± 1.7	14 ± 1.3
					500	15 ± 1.2	16 ± 0.9	134 ± 11.3	122 ± 15.5	16 ± 1.2	13 ± 1.8
					5000	22 ± 3.9	19 ± 1.8	125 ± 16.9	155 ± 10.7	16 ± 0.6	11 ± 1.5
Euphorbiaceae	*Spirostachys africana* Sond.	Leaves & Twigs	SNR	S. Amoo 25 NU[b]	50	17 ± 0.7	23 ± 1.2	162 ± 6.4	154 ± 2.5	11 ± 2.3	15 ± 2.0
					500	19 ± 0.9	20 ± 1.53	181 ± 17.7	151 ± 13.2	12 ± 1.5	15 ± 1.2
					5000	21 ± 2.2	18 ± 1.2	172 ± 14.6	164 ± 12.5	13 ± 2.1	13 ± 0.9
Lamiaceae	*Tetradenia riparia* (Hochst.) Codd	Leaves	UKZN	S. Amoo 20 NU[a]	50	19 ± 2.4	15 ± 1.8	168 ± 4.1	163 ± 3.2	13 ± 1.2	14 ± 0.3
					500	21 ± 3.6	18 ± 1.8	181 ± 6.4	183 ± 5.4	10 ± 0.9	13 ± 1.5
					5000	17 ± 0.7	23 ± 1.5	156 ± 6.9	181 ± 16.0	10 ± 1.7	10 ± 1.2
Lauraceae	*Ocotea bullata* (Burch.) Baill.	Bark	UKZN	S. Amoo 13 NU[a]	50	20 ± 3.2	19 ± 1.7	142 ± 11.7	148 ± 11.5	14 ± 0.6	11 ± 1.5
					500	19 ± 1.3	18 ± 0.9	172 ± 6.2	156 ± 15.6	16 ± 1.5	15 ± 2.9
					5000	19 ± 3.6	19 ± 2.0	178 ± 7.0	155 ± 9.8	15 ± 0.9	13 ± 1.9
Malvaceae	*Dombeya rotundifolia* Hochst.	Leaves	UKZN	S. Amoo 11 NU[b]	50	21 ± 6.7	19 ± 0.3	206 ± 9.8	144 ± 9.2	14 ± 1.8	11 ± 0.6
					500	17 ± 0.6	14 ± 1.5	171 ± 2.6	141 ± 5.6	14 ± 2.3	14 ± 2.0
					5000	21 ± 2.6	23 ± 5.0	194 ± 4.4	148 ± 10.4	13 ± 1.9	16 ± 4.1
Meliaceae	*Ekebergia capensis* Sparrm	Leaves & Twigs	SNR	S. Amoo 22 NU[a]	50	21 ± 1.8	16 ± 1.3	165 ± 6.8	194 ± 17.9	11 ± 0.6	10 ± 0.6
					500	18 ± 1.3	16 ± 1.5	167 ± 8.3	169 ± 12.6	16 ± 1.5	13 ± 1.8
					5000	20 ± 1.7	20 ± 3.0	171 ± 19.4	194 ± 16.1	14 ± 1.2	13 ± 0.6
Rhamnaceae	*Ziziphus mucronata* Willd.	Leaves	SNR	S. Amoo 17 NU[a]	50	22 ± 3.3	14 ± 0.9	141 ± 14.2	148 ± 3.8	11 ± 0.7	14 ± 2.7
					500	20 ± 2.4	18 ± 1.4	171 ± 16.9	165 ± 8.1	13 ± 0.7	12 ± 2.0
					5000	19 ± 2.0	14 ± 1.3	152 ± 4.7	163 ± 7.7	13 ± 1.5	14 ± 1.2
Rutaceae	*Clausena Anisata* (Willd.) Hook. F. ex Benth	Leaves & Twigs	UKZN	S. Amoo 18 NU[b]	50	18 ± 3.2	17 ± 1.2	179 ± 9.0	149 ± 12.5	12 ± 1.7	16 ± 0.1
					500	12 ± 1.2	19 ± 1.7	185 ± 17.1	141 ± 10.3	10 ± 0.3	12 ± 3.7
					5000	17 ± 1.5	17 ± 1.2	157 ± 10.0	167 ± 4.6	12 ± 1.5	14 ± 0.7
Solvent control (50% methanol)						15 ± 1.5		166 ± 12.0		14 ± 2.0	
Positive control (4-nitroquinoline-1-oxide at 2 μg/plate)						294 ± 3.9		1583 ± 71.8		38 ± 3.7	

Values are expressed as mean ± standard error where n = 6.
SNR = Silverglen Nature Reserve, Durban; UKZN = University of KwaZulu-Natal Botanical Garden, Pietermaritzburg.
[a] Voucher number of plant material stored for 16 years was as described by Jäger et al. (1996).
[b] Voucher number of plant material stored for 12 years was as described by McGaw et al. (2000).

effects, the same set of plant materials was collected from the same location and during the same season as the stored ones. The collected plant materials were oven-dried at 50 °C and stored at room temperature (25 °C) in dark conditions for 12 or 16 years. The freshly harvested materials were similarly oven-dried at 50 °C. After identification by Dr C. Potgieter, voucher specimens were lodged in The Bews Herbarium, University of KwaZulu-Natal, Pietermaritzburg, South Africa.

For both stored and freshly collected plant species, the oven-dried materials were ground into fine powders through a 1 mm ring sieve using an Ultra Centrifugal Mill (ZM 200, Retsch®, Germany). Plant materials (1 g in 20 ml) were extracted with 50% methanol in a sonication bath (Julabo GmbH, West Germany) containing ice-cold water for 1 h. Extracts were filtered through Whatman No.1 filter paper and concentrated in vacuo using a rotary evaporator (Büchi, Switzerland) at 40 °C. The concentrates were transferred to pre-weighed glass pill vials and completely dried under a stream of air. Dried extracts were dissolved in 50% methanol at a concentration of 5000 μg/ml and filtered through sterile 0.22 μm filters. Thereafter, two lower concentrations of 500 and 50 μg/ml were prepared.

2.2. Ames assay

Mutagenic potential of the extracts from the stored and freshly collected plant materials was evaluated using the Ames *Salmonella*/microsome mutagenicity assay, involving three *Salmonella typhimurium* tester strains (TA98, TA100, TA1535) in the absence of metabolic activation (Maron and Ames, 1983; Mortelmans and Zeiger, 2000). For each *S. typhimurium* tester strain, 100 μl of the bacteria was inoculated in 10 ml of Oxoid nutrient broth No. 2 for 16 h at 37 °C to obtain a density of approximately 1×10^9 colony forming units per millilitre (CFU/ml). In sterile test tubes (triplicate), 100 μl of the prepared sample was added to 500 μl phosphate buffer (0.1 mM, pH 7.4) followed by 100 μl of overnight bacteria culture. Finally, 2 ml of melted top agar (containing 10 mM histidine maintained at 50 °C) was added to the mixture in sterile test tubes. After vortexing, the test tube content was poured in minimal agar plates and allowed to solidify (2–3 min). The solvent control consisted of 50% methanol (100 μl/plate) while 4-nitroquinoline-N-oxide (4-NQO; Fluka Chemika, Switzerland) at 2 μg/plate was used as a positive control. After incubation at 37 °C for 48 h, the colonies were counted using a colony counter (Anderman, UK). The assay was conducted twice and results are expressed as a mean (± standard error) number of reverted colonies per plate. An extract was classified as a mutagen if (i) the number of histidine (his^+) revertants was at least double that of the negative control and (ii) when there was a dose dependent increase in the number of his^+ revertants (Cariello and Piegorsch, 1996).

3. Results and discussion

Based on the sensitivity, simplicity and affordability of the Ames test, the assay remains one of the most common and widely accepted methods for assessing the potential mutagenicity in several substances and compounds (Mortelmans and Zeiger, 2000; Taylor et al., 2003; Verschaeve and Van Staden, 2008). As an indication of the potential mutagenicity of the evaluated medicinal plants, the number of his^+ revertants obtained at 50, 500 and 5000 μg/ml for all the extracts is shown in Table 1. Apart from the freshly collected *Acokanthera oppositifolia* extract at 5000 μg/ml which exhibited mutagenic effects against TA1535 tester strain, all the other plant extracts did not show any mutagenic effect against the three tester bacterial strains. Mutagenic potential observed with TA1535 tester is associated with the substitution of leucine with proline in the bacterial genome (Mortelmans and Zeiger, 2000). Generally, a positive response in any single bacterial strain either with or without metabolic activation is sufficient to designate a substance as a mutagen (Zeiger, 2001). Therefore, necessary precautions must be exercised with the use of high concentration of *A. oppositifolia* especially when freshly collected. It is known that mutagenic substances or compounds could potentially damage the germ line leading to fertility problems and to mutations in future generations and may induce cancer (Mortelmans and Zeiger, 2000). The effect of storage on the mutagenicity of a potential mutagen remains poorly understood in view of the limited number of available studies (Pagano and Zeiger, 1985). As a result, it is difficult to accurately explain the non-mutagenic effect of *A. oppositifolia* after prolonged storage, as observed in the current study. Perhaps, the breakdown of constituent chemicals (Stafford et al., 2005) in the medicinal plants after storage resulted in the current observation. Indeed, there was a significant higher level of secondary metabolites such as total phenolics, flavonoids and gallotannins in freshly collected *A. oppositifolia* extract compared to the stored one (Amoo et al., 2012a). Although it is difficult to establish a particular relationship between the higher level of flavonoid content and the observed mutagenicity in freshly harvested *A. oppositifolia*, the likelihood that some of the flavonoids present are mutagenic cannot be eliminated. In fact, certain flavonoids, including quercetin, galangin and kaempferol are mutagenic in some *S. typhimurium* strains as shown by the Ames test (Boersma et al., 2000; Resende et al., 2012).

Toxicology testing is an essential requirement for the development of modern pharmaceutical drugs. Such tests are also important for medicinal plants. Therefore, the possible bacterial toxicity was assessed by observing the background lawn of bacterial growth. The high likelihood of the absence of toxicity in the evaluated medicinal plants (both stored and fresh) was established with the presence of a granular thin film layer on the background lawn (Mortelmans and Zeiger, 2000). In addition, the number of his^+ revertants observed in each of the tester strains in any of the medicinal plants evaluated was not much lower (perhaps half) than what was recorded in the solvent control.

4. Conclusions

Taken together, the current findings add to the available information on the safety of medicinal plant consumption. The safety of medicinal plants is one of the core issues of concern in the integration of traditional medicine into primary health care systems. The current study indicates the absence of any mutagenic effect resulting from the storage of medicinal plants for a long time. In fact, the stored *A. oppositifolia* was devoid of any mutagenic effect whereas the freshly collected material demonstrated potential mutagenic effect against one of the tester strains used. It seems logical to conclude that the majority of these medicinal plants may be safe for use even after prolonged storage. Nevertheless, further studies involving a battery of tests as well as the use of S9 metabolic activation system and *in vivo* conditions may further elucidate the effect of long-term storage on the safety of medicinal plants.

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References

Alves, R., Rosa, I., 2007. Biodiversity, traditional medicine and public health: where do they meet? Journal of Ethnobiology and Ethnomedicine 3, 14.

Amoo, S.O., Aremu, A.O., Moyo, M., Van Staden, J., 2012a. Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. BMC Complementary and Alternative Medicine 12, 87.

Amoo, S.O., Aremu, A.O., Moyo, M., Van Staden, J., 2012b. Assessment of long-term storage on antimicrobial and cyclooxygenase-inhibitory properties of South African medicinal plants. Phytotherapy Research. http://dx.doi.org/10.1002/ptr.4830.

Boersma, M.G., Vervoort, J., Szymusiak, H., Lemanska, K., Tyrakowska, B., Cenas, N., Segura-Aguilar, J., Rietjens, I.M.C.M., 2000. Regioselectivity and reversibility of the glutathione conjugation of quercetin quinone methide. Chemical Research in Toxicology 13, 185–191.

Cariello, N.F., Piegorsch, W.W., 1996. The Ames test: the two-fold rule revisited. Mutation Research/Genetic Toxicology 369, 23–31.

Fennell, C.W., Light, M.E., Sparg, S.G., Stafford, G.I., Van Staden, J., 2004a. Assessing African medicinal plants for efficacy and safety: agricultural and storage practices. Journal of Ethnopharmacology 95, 113–121.

Fennell, C.W., Lindsey, K.L., McGaw, L.J., Sparg, S.G., Stafford, G.I., Elgorashi, E.E., Grace, O.M., Van Staden, J., 2004b. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. Journal of Ethnopharmacology 94, 205–217.

Griggs, J.K., Manandhar, N.P., Towers, G.H.N., Taylor, R.S.L., 2001. The effects of storage on the biological activity of medicinal plants from Nepal. Journal of Ethnopharmacology 77, 247–252.

Gurib-Fakim, A., 2006. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine 27, 1–93.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A., 1996. Zulu Medicinal Plants. An Inventory. University of Natal Press, Pietermaritzburg, South Africa.

Jäger, A.K., Hutchings, A., Van Staden, J., 1996. Screening of Zulu medicinal plants for prostaglandin-synthesis inhibitors. Journal of Ethnopharmacology 52, 95–100.

Mander, M., Ntuli, L., Diederichs, N., Mavundla, K., 2007. Economics of the traditional medicine trade in South Africa. In: Harrison, S., Bhana, R., Ntuli, A. (Eds.), South African Health Review. Health Systems Trust, Pretoria, South Africa, pp. 189–200.

Maron, D.M., Ames, B.N., 1983. Revised methods for the *Salmonella* mutagenicity test. Mutation Research/Environmental Mutagenesis and Related Subjects 113, 173–215.

McGaw, L.J., Jäger, A.K., Van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. Journal of Ethnopharmacology 72, 247–263.

Mortelmans, K., Zeiger, E., 2000. The Ames *Salmonella*/microsome mutagenicity assay. Mutation Research, Fundamental and Molecular Mechanisms of Mutagenesis 455, 29–60.

Newman, D.J., Cragg, G.M., Snader, K.M., 2003. Natural products as sources of new drugs over the period 1981–2002. Journal of Natural Products 66, 1022–1037.

Pagano, D.A., Zeiger, E., 1985. The stability of mutagenic chemicals stored in solution. Environmental Mutagenesis 7, 293–302.

Resende, F.A., Vilegas, W., dos Santos, L.C., Varanda, E.A., 2012. Mutagenicity of flavonoids assayed by bacterial reverse mutation (Ames) test. Molecules 17, 5255–5268.

Rybicki, E.P., Chikwamba, R., Koch, M., Rhodes, J.I., Groenewald, J.-H., 2012. Plant-made therapeutics: an emerging platform in South Africa. Biotechnology Advances 30, 449–459.

Stafford, G.I., Jäger, A.K., Van Staden, J., 2005. Activity of traditional South African sedative and potentially CNS-acting plants in the GABA-benzodiazepine receptor assay. Journal of Ethnopharmacology 100, 210–215.

Street, R.A., Stirk, W.A., Van Staden, J., 2008. South African traditional medicinal plant trade — challenges in regulating quality, safety and efficacy. Journal of Ethnopharmacology 119, 705–710.

Taylor, J.L.S., Elgorashi, E.E., Maes, A., Van Gorp, U., De Kimpe, N., Van Staden, J., Verschaeve, L., 2003. Investigating the safety of plants used in South African traditional medicine: testing for genotoxicity in the micronucleus and alkaline comet assays. Environmental and Molecular Mutagenesis 42, 144–154.

Van Wyk, B.-E., 2011. The potential of South African plants in the development of new medicinal products. South African Journal of Botany 77, 812–829.

Verschaeve, L., Van Staden, J., 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. Journal of Ethnopharmacology 119, 575–587.

Wiersum, K.F., Dold, A.P., Husselman, M., Cocks, M., 2006. Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the Amatola region, South Africa. In: Bogers, R.J., Craker, L.E., Lange, D. (Eds.), Medicinal and Aromatic Plants. Springer, Nertherlands, pp. 43–57.

Zeiger, E., 2001. Mutagens that are not carcinogens: faulty theory or faulty tests? Mutation Research, Genetic Toxicology and Environmental Mutagenesis 492, 29–38.